PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Loss of GD1-positive Lactobacillus correlates with inflammation in human lungs with COPD
AUTHORS	Sze, Marc; Utokaparch, Soraya; Elliott, Mark; Hogg, James; Hegele, Richard

VERSION 1 - REVIEW

REVIEWER	Alejandro Pezzulo University of Iowa Hospitals and Clinics U.S.A
REVIEW RETURNED	15-Oct-2014

GENERAL COMMENTS	Sze et al. have submitted a simple yet elegant study in which they hypothesized that lungs of people with COPD would contain different bacterial populations than lungs of healthy people. Their context is provided by data previously published by the same group in which they demonstrated that COPD GOLD 4 lungs had higher amounts of Lactobacillus, and by studies demonstrating that GD1 positive Lactobacillus reduced inflammation in an albumin challenge model in mice. The main advantage in their approach comes from their ability to analyze lung tissue removed surgically under sterile conditions. This avoids the oropharynx/upper airway contamination bias plaguing most studies of airway microbiome. Using their combination of quantitative histology and high throughput bacterial sequencing, they found that: 1.- COPD GOLD 1/2 lungs do not contain different amounts of either total bacteria or Lactobacillus DNA. 2.- The difference in GD1 (+) Lactobacillus in COPD lungs correlates inversely with degree of inflammation. They then use their findings to conclude that GD1 (+) Lactobacillus loss may contribute to COPD pathogenesis. ***Minor Revisions*** 1.- I would suggest changing the title of the manuscript to more accurately reflect the findings. i.e., “Loss of GD1-positive Lactobacillus correlates with inflammation in human lungs with COPD” makes it easier to immediately understand the study than the current -perhaps vague- title. ABSTRACT: 2.- (line 8): Please change “the status of GD1 positivity” to “contents of GD1-positive Lactobacillus” 3.- (line 34): Please include the fact that your samples are not confounded by sampling through the mouth (a strength of your study) 4.- (line 41): A small sample size is only a problem if you require a larger sample size to achieve a certain statistical power… you may benefit from running a post-hoc power analysis to quickly list in your

	methods section how much discriminating function your study had. This is relevant only for outcomes that were not statistically significantly different in control vs. study samples. This is a minor comment that I do not think has to result in changes but would make it clearer why a small sample may be a problem. RESULTS/LEGENDS/FIGURES: 5.- My only suggestion here is to change the Y axis of the figures in Figure 1 to express " % GD1 positive" in both cases. The current form in which the data from the GOLD4 samples is shown as total number whereas the newer data is shown as percentage is confusing, as is the label for the Y axis ("distribution"). DISCUSSION: 6.- The authors have pointed out the main flaws and strengths of their study adequately and have put their results in the context of recent data. I do think that they also need to point out that it is always hard to determine whether their findings regarding GD1 are cause or consequence of lung damage, as is the case with most of the current literature analyzing microbiomes. I find their new hypothesis that reuterin may have a causal relationship with COPD inflammation particularly compelling and would perhaps emphasize this a bit more in the discussion.

REVIEWER	Christian Taube LUMC The Netherlands No competing interests declared
REVIEW RETURNED	30-Dec-2014

GENERAL COMMENTS	In the present study the authors investigate the relationship between the detection of lactobacillus in lung samples from patients with lung cancer, comparing patients with COPD (GOLD I and II) and smokers. In addition historic samples from a previous study are investigated containing non-smokers, smokers and COPD stage IV patients. Analysis of microbial colonization in the lung is of major interest and indeed the present paper offer some more insight. However, there are a couple of concerns. 1. The authors find no difference in the abundance of lactobacilli in the group of COPD patients, which is overall not high (between 2.5 and 8.7% of the groups). In addition, they find from this small number of lactobacillus positive patients only a small fraction in the smoking control group (in total 5 patients of the 74). However, the title and the discussion suggest that this factor may play a quite important role. Overall, I think these findings are interesting and it is worthwhile to describe the situation in COPD patients. However given the rarity of lactobacillus in the lung especially of the GD1 producing strain I would rather suggest to be more descriptive and stress not too much a potential clinical relevance. 2. The authors compare 5 patients without GD1 producing lactobacilli in the lung to the rest (mixture of COPD I, II and controls) in terms of inflammation. In my opinion this is not a justified comparison as the authors compare the controls (where 4 of the 5 GD1 positive patients are from) to COPD patients where we know that increase inflammation can be detected around the airways. 3. Is data available on the composition of other microorganisms in

	the lung of these patients? Is the presence/absence of lactobacillus associated with an increase/decrease in other species?

VERSION 1 – AUTHOR RESPONSE

Reviewer 1: Alejandro Pezzulo

1.- I would suggest changing the title of the manuscript to more accurately reflect the findings. i.e., "Loss of GD1-positive Lactobacillus correlates with inflammation in human lungs with COPD" makes it easier to immediately understand the study than the current -perhaps vague- title.

R1: We have changed the title of the manuscript to reflect your suggestion.

ABSTRACT:

2.- (line 8): Please change "the status of GD1 positivity" to "contents of GD1-positive Lactobacillus"

R2: Respective change has been made.

3.- (line 34): Please include the fact that your samples are not confounded by sampling through the mouth (a strength of your study)

R3: This point has been added to the strength and limitations section of the manuscript.

4.- (line 41): A small sample size is only a problem if you require a larger sample size to achieve a certain statistical power… you may benefit from running a post-hoc power analysis to quickly list in your methods section how much discriminating function your study had. This is relevant only for outcomes that were not statistically significantly different in control vs. study samples. This is a minor comment that I do not think has to result in changes but would make it clearer why a small sample may be a problem.

R4: Thank you for this comment. We have modified the abstract so that the comment about larger numbers is taken out and replaced with "…validation of these results need to be completed before…" However we prefer not to make any changes in the body of the manuscript itself.

RESULTS/LEGENDS/FIGURES:

5.- My only suggestion here is to change the Y axis of the figures in Figure 1 to express " % GD1 positive" in both cases. The current form in which the data from the GOLD4 samples is shown as total number whereas the newer data is shown as percentage is confusing, as is the label for the Y axis ("distribution").

R5: This change has been made in the revised manuscript.

6.- The authors have pointed out the main flaws and strengths of their study adequately and have put their results in the context of recent data. I do think that they also need to point out that it is always hard to determine whether their findings regarding GD1 are cause or consequence of lung damage, as is the case with most of the current literature analyzing microbiomes. I find their new hypothesis that reuterin may have a causal relationship with COPD inflammation particularly compelling and would perhaps emphasize this a bit more in the discussion.

R6: We have added a comment in the discussion about the need for potential in vitro studies to try and work out potential cause and effect (end of second to last paragraph in the discussion, Paragraph 5)

Reviewer 2: Christian Taube

1. The authors find no difference in the abundance of lactobacilli in the group of COPD patients, which is overall not high (between 2.5 and 8.7% of the groups). In addition, they find from this small number of lactobacillus positive patients only a small fraction in the smoking control group (in total 5 patients of the 74). However, the title and the discussion suggest that this factor may play a quite important role. Overall, I think these findings are interesting and it is worthwhile to describe the situation in COPD patients. However given the rarity of lactobacillus in the lung especially of the GD1 producing strain I would rather suggest to be more descriptive and stress not too much a potential clinical relevance.

R1: We have changed the title to what was suggested by reviewer 1.

2. The authors compare 5 patients without GD1 producing lactobacilli in the lung to the rest (mixture of COPD I, II and controls) in terms of inflammation. In my opinion this is not a justified comparison as the authors compare the controls (where 4 of the 5 GD1 positive patients are from) to COPD patients where we know that increase inflammation can be detected around the airways.

R2: This is a very good point and we have included in the supplement how the volume fraction measurements breakdown by GOLD grade. There is more inflammation in the GOLD 1 samples that we measured but there is no difference between control and GOLD 2 samples [Figure S7]. When we compare only the control samples by GD1 positivity the significant difference holds for the macrophage measurement [Figure S8]. In contrast, although a trend still exists, no difference was found for the PMN measurement [Figure S9]. We have added a reference to these three figures in the result section of the manuscript.

3. Is data available on the composition of other microorganisms in the lung of these patients? Is the presence/absence of lactobacillus associated with an increase/decrease in other species?

R3: At the moment data is not available on the other microorganisms but we hope to be able to eventually catalog more species specific information within these samples at a later date. The question of whether or not other species are correlated with increases or decreases of Lactobacillus is a good question that we hope to answer eventually. At the moment this question is outside the scope of this particular study.